U.S.S.N.: 09/448,042 Filed: November 23, 1999 Response, page 11



USSN 09/09/448,042

Clean Copy of Pending Claims

- 1. (Twice Amended) A method for determining the presence of a target nucleotide, the method comprising the steps of:
- (a) exposing a biological sample selected from the group consisting of blood, stool and urine to a nucleic acid primer capable of hybridizing with a nucleic acid, said primer having a covalently-attached donor molecule comprising a fluorophore or a fluorescent dye;
- (b) performing a primer extension reaction in the presence of a dideoxy nucleotide complementary to the target nucleotide, said dideoxy nucleotide having a covalently-attached acceptor molecule comprising a fluorophore or a fluorescent dye, said acceptor molecule being capable of being activated through fluorescent energy transfer from said donor molecule so as to produce a detectable fluorescent signal when said dideoxy nucleotide is incorporated into a product resulting from the primer extension reaction;
- (c) determining the presence of said fluorescent signal, said presence being indicative of incorporation of said dideoxy nucleotide into the primer extension product; and
- (d) determining the presence of said target nucleotide as indicated by the incorporation of said dideoxy nucleotide into the primer extension product.
- 4. The method of claim 1, wherein said extension reaction is performed in the presence of at least two different dideoxy nucleotides, each comprising a different acceptor molecule that produces a distinct fluorescent signal upon activation.
- 10. (Twice Amended) The method of claim 1, wherein said fluorescent dye is

U.S.S.N.: 09/448,042 Filed: November 23, 1999 Response, page 12



selected from the group consisting of 6-carboxyfluorescein (FAM), 6-carboxy-X-rhodamine (REG), N₁, N₁ N¹, N¹-tetramethyl-6-carboxyrhodamine (TAMARA), 6-carboxy-X-rhodomine (ROX), fluorescein, Cy5® and LightCycler-Red 640.

- 11. The method of claim 1 wherein said donor molecule comprises 6-carboxyfluorescein (FAM).
- 12. The method of claim 11 wherein said acceptor molecule comprises, 6-carboxy-X-rhodomine (ROX).
- 15. The method of claim 1 wherein said dideoxy nucleotide is a 2'3 '-dideoxy nucleotide triphosphate selected from the group consisting of ddATP, ddCTP, ddGTP, ddTTP and ddUTP.
- 18. The method of claim 1, wherein said target nucleotide is present as a result of a nucleic acid mutation.
- 19. (Amended) The method of claim 18, wherein said mutation occurs in a gene selected from the group consisting of ras oncogenes, p53, dcc, apc, mcc and β-catenin.
- 20. The method of claim 4, wherein said target nucleotide is present at a single nucleotide polymorphic locus.
- 25. (New) A method for determining the presence of a target nucleotide, the method comprising the steps of:
- (a) exposing a biological sample comprising a bodily fluid to a nucleic acid primer capable of hybridizing with a nucleic acid, said primer having a covalently-attached donor molecule comprising a fluorophore or a fluorescent dye;
- (b) performing a primer extension reaction in the presence of a dideoxy nucleotide complementary to the target nucleotide, said dideoxy nucleotide having a covalently-



U.S.S.N.: 09/448,042 Filed: November 23, 1999

Response, page 13

attached acceptor molecule comprising a fluorophore or a fluorescent dye, said acceptor molecule being capable of being activated through fluorescent energy transfer from said donor molecule so as to produce a detectable fluorescent signal when said dideoxy nucleotide is incorporated into a product resulting from the primer extension reaction;

- (c) determining the presence of said fluorescent signal, said presence being indicative of incorporation of said dideoxy nucleotide into the primer extension product; and
- (d) determining the presence of said target nucleotide as indicated by the incorporation of said dideoxy nucleotide into the primer extension product.
- 26. (New) The method of claim 25, wherein said bodily fluid is selected from the group consisting of pus, semen, sputum, saliva, cerebrospinal fluid, biopsy tissue and lymph.
- 27. (New) The method of claims 1 or 25, wherein said biological sample is obtained from a pooled patient population.